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COMMENTARY

Immunology and efficacy of MF59-adjuvanted vaccines

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ABSTRACT

Adjuvants are included in vaccine formulations to enhance the immunogenicity and efficacy of vaccines. MF59® is an oil-in-water emulsion adjuvant and licensed for use in pandemic and seasonal influenza vaccines in many countries. MF59 is safe and well tolerated in humans. MF59-adjuvanted vaccination spares vaccine dose and enhances hemagglutination inhibiting antibodies against homologous and heterologous influenza virus strains. The mechanisms of MF59 involve rapid induction of chemokines, inflammatory cytokines, recruiting multiple immune cells, uric acid and benign apoptosis of certain innate immune cells. The adjuvant effects of MF59 on generating vaccine-specific isotype-switched IgG antibodies, effector CD8 T cells, and protective immunity were retained even in a CD4-deficient condition by inducing effective immune-competent microenvironment with various innate and antigen presenting cells in a mouse model. CD4-independent adjuvant effects of MF59 might contribute to improving the vaccine efficacy in children, the elderly, and immunocompromised patients as well as in healthy adults. Further studies will be needed to broaden the use of MF59 in various vaccine antigens and populations as well as lead to better understanding of the action mechanisms of MF59 adjuvant.

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Introduction

MF59® was approved to be included in human influenza vaccines in over 30 countries for use in individuals including children and the elderly.[1–3] MF59 is an oil-in-water emulsion containing squalene (4.3%) in citric acid buffer with stabilizing nonionic surfactants Tween 80 (0.5%) and Span 85 (0.5%). Squalene is naturally synthesized in the pathway of human steroid hormones, and present in skin, adipose tissue and muscles. The natural squalene is obtained from shark liver and prepared for vaccine adjuvants after purification. Also, Tween 80 and Span 85 are plant-derived pharmaceutical surfactants. All components of MF59 are biodegradable natural derivatives, safe, and well tolerated. The mean size of the oil droplets is approximately 160 nm. MF59 was originally developed as an antigen delivery buffer and tested first with an antigen and immune potentiator, but surprisingly potent adjuvant effects were discovered in MF59 formulation itself.[2]

A MF59-adjuvanted seasonal influenza vaccine (Fluad®) was first licensed in 1997 for the elderly, and since then have been approved to be included in human vaccines in over 30 countries including the US.[3] In addition to seasonal influenza vaccine, MF59-adjuvanted H1N1 pandemic influenza vaccine (Focetria® and Celtura®) has been distributed to populations including pregnant women and young children with approximately 100 million doses.[2] MF59 adjuvant effects on influenza vaccination include increased immunogenicity of vaccines such as hemagglutination inhibiting (HAI) antibodies and memory T and B cells against antigenically drifted influenza viruses, resulting in more effective pandemic and seasonal influenza vaccines.[4,5]

A common feature of many adjuvants included in vaccination is the rapid induction of innate immune responses at the site of injection and draining lymph nodes, which is needed for effective antigen presentation to CD4 T helper cells providing immunological help to develop B cells and CD8 T cell adaptive immunity.[1,3] Vaccine adjuvants are considered to play a key role in educating CD4 T helper cells via generating inflammatory innate immune responses and activating antigen presenting cells (APCs). Here, we review the MF59 adjuvant effects in a CD4-dependent and CD4-independent manner and their implications for adjuvanted vaccination.

CD4-dependent and CD4-independent adjuvant effects of MF59

Both antigen and MF59 were shown not to be detected within a few hours after injection in the experiments using radiolabeled or fluorescence-labeled antigen and MF59.[6] MF59 recruited immune cells in C-C motif chemokine receptor 2 [7] and intercellular adhesion molecule-1[8] dependent manner, and chemokines such as C-C motif chemokine ligands (CCL) 2, CCL3, CCL4 and interleukin (IL)-8 were secreted by MF59-treated cells to recruit more immune cells at the site of injection.[9] Antigens and MF59 are taken up by neutrophils and monocytes, and later followed by dendritic cells (DCs) and B cells, and moved to draining lymph nodes.[1,10] Recently, MF59 was shown to promote differentiation of monocyte-derived DCs (Mo-DCs) within draining lymph nodes and these Mo-DCs were the major APCs to enhance antigen-

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specific CD4 T cell responses.[11] In addition, MF59 enhanced CD4 T follicular helper cell activation and following germinal center reaction to increase B cell responses in mice.[12] CD4 T cells are required for inducing B cell responses to generate isotype-switched IgG antibodies to subunit split virus or protein antigen vaccination.[13] A conventional view is that vaccine adjuvants activate the innate immune system including APCs, mediate the induction of appropriate T helper cell responses such as T helper (Th) 1, Th2 and Th17, which determines the quality and quantity of antigen-specific B cell responses and the outcomes of adaptive immunity.[14–16]

In our recent study, inactivated split influenza vaccine failed to induce IgG antibody responses in CD4 deficient mice, suggesting that split influenza vaccine is T-dependent antigen and requires CD4 T helper cells to induce antigen specific IgG antibody responses.[13] As expected, the adjuvant effects of alum require the presence of CD4 T helper cells in the context of T-dependent split influenza vaccine. In contrast to the CD4 T cell-dependent adjuvant effects of alum, inclusion of MF59 in split influenza vaccination of CD4 deficient mice or CD4-depleted wild type mice mediated the induction of IgG isotype-switched antibodies at the levels comparable to those in wild type mice.[13] In addition, the MF59 adjuvant effects in CD4 deficient mice include the induction of HAI antibodies, long-lived antibody secreting plasma cells in bone marrow, protective CD8 T cell responses, and complete protection against lethal influenza infection.[13] Thus, MF59 can exhibit the adjuvant effects on promoting IgG isotype-switching and enhancing adaptive immunity after vaccination in a CD4-deficient condition, suggesting a CD4-independent pathway connecting innate immune responses and adaptive immune systems in addition to the conventional CD4 T helper cell-dependent mechanisms.[13] In contrast to the independence of CD4 T cell help, the expression of major histocompatibility complex class II molecule (MHCII) was required for the adjuvanticity of MF59. Mechanisms of CD4-independent adjuvant effects of MF59 remain to be further determined.

Peritoneal injection of MF59 was effective in acute induction of inflammatory cytokines (IL-6, tumor necrosis factor-alpha), chemokine (CCL5), eosinophils, DC subsets, and natural killer (NK) T cells in CD4 deficient mice, at higher levels than those in wild type mice.[13] Other cytokine (IL-5) and chemokines (Monocyte chemoattractant protein 1 [MCP-1]), monocytes, neutrophils, and NK cells were induced at comparable levels in CD4 deficient and wild type mice after MF59 injection, which were higher levels than alum adjuvant.[13] Signaling from cell death induces local inflammatory microenvironment triggering the innate immune activation and promotes the adaptive immune responses.[17] MF59 induces non-harmful apoptosis of DCs in draining lymph nodes after intramuscular injection.[7] Peritoneal injection of MF59 in mice induced depletion of macrophages and DCs and increased uric acid which is a danger signal released after cell death at the site of injection.[13] Interestingly, MF59 but not alum adjuvant could recruit various innate immune cells (monocytes, neutrophils, eosinophils) and APCs ($CD11b^{high/low}$ DCs) at the site of injection in CD4-deficient mice.[13] In line with *in vivo* effects, *in vitro* cultures of bone marrow derived DCs, macrophages and DC2.4 DC cell lines with MF59 treatment induced both apoptosis and necrotic cell death.[13] MF59 does not activate any of the Toll-like receptors (TLR) *in vitro*, but its adjuvanticity requires myeloid differentiation primary response 88 (MyD88), suggesting MF59 adjuvant effects by a TLR-independent signaling pathway.[18] The *in vivo* adjuvant effects of MF59 were shown to require the roles of the apoptosis-associated speck-like protein containing a caspase recruitment domain, IL-4 and Stat-6 signaling but independently of type-1 interferon and inflammasome signaling pathways.[19–21]

MF59 in comparison with other adjuvants

Compared to alum, MF59-adjuvanted influenza vaccine induced higher levels of antigen-specific antibody production, higher HAI titers, and better protection in a mouse model.[13,22,23] In addition, MF59 with different vaccine antigens (tetanus toxoid, hepatitis B, Group B and C Meningococcal bacteria) has also shown better adjuvant efficacy than alum adjuvant in mice.[24] MF59 was more potent in rapid induction of inflammatory cytokines (IL-5), chemokine (MCP-1), uric acid, and in recruiting innate immune cells (monocytes, neutrophils, NK cells, lymphocytes) compared to alum.[1,10,13] In clinical studies, MF59-adjuvanted vaccination increased HAI titers by 2 to 5 folds when compared to those by alum-adjuvanted vaccination with A/H5N1 influenza subunit vaccine.[25]

Adjuvant system 04 (AS04) was developed and licensed by GlaxoSmithKline Biologicals and has been used in hepatitis B virus vaccine (Fendrix) and human papillomavirus vaccine (Cervarix). It is a combination of alum and monophosphoryl lipid A (MPL), a TLR4 agonist. The immune stimulation effects of AS04 were mainly due to MPL, and it induces increased APC activation and local nuclear factor (NF)-kappaB activity and cytokine production. And alum in AS04 appears to prolong the cytokine responses of MPL at the site of injection.[26] Both MF59 and AS04 adjuvanted influenza vaccinations were effective in inducing IgG isotype-switched antibodies and conferring protective immunity in a CD4 deficient mouse model.[13,27] MF59 was more potent than AS04 in exhibiting CD4-independent adjuvant effects. AS04 showed a moderate level of CD4-dependency in inducing isotype-switched IgG antibodies, but AS04-adjuvanted T-dependent split influenza vaccine provided sufficient protection in CD4-deficient mice. Both MF59 and AS04 appeared to generate local inflammatory microenvironment and recruit DCs at the site of injection. In addition to APCs, CD8 T cells and double negative T cells were increased in MF59 or AS04-treated CD4 deficient mice. MHCII-expressing cellular components, double negative T cells, and soluble cytokines and chemokines by MF59 or AS04 adjuvants are likely to be the major contributing factors in providing alternative help to B cells for inducing IgG antibody responses in a CD4-deficient condition.

Clinical applications of MF59 vaccine adjuvant

In a healthy adult population, MF59-adjuvanted low-dose influenza A/H5N1 vaccination induced higher HAI titers than those of high doses of unadjuvanted vaccination.[28] High titers of cross-reactive antibodies were rapidly induced

and remained detectable among MF59-adjuvatned pre-pandemic H5 vaccine primed subjects.[4] Also, MF59-adjuvanted single low-dose (3.75 μg) influenza A/H1N1 vaccine induced optimal immune responses in young to middle-aged (18–64 years) and older (≥ 65 years) adult populations, and higher vaccine doses with MF59 induced highest antibody titers.[29] MF59 adjuvanted influenza A/H1N1 vaccine and seasonal trivalent vaccines display benefits of antigen dose sparing, higher antibody responses, longer antibody maintenance, and magnitude of innate and adaptive immune responses in young children and adolescents.[30–32] In particular, MF59-adjuvanted influenza vaccine provided immune responses to heterologous strain, greater protection, and clinical benefits in vaccine-naïve children aged 6 months through 5 years.[21,33] In retrospective investigations of pregnant women vaccinated with Focetria (MF59-adjuvanted pandemic A/H1N1), there was no statistically significant association of maternal, fetal and neonatal outcomes between adjuvanted and unadjuvanted vaccine-administered cohorts, suggesting that MF59 adjuvanted A/H1N1 pandemic influenza vaccination was safe during pregnancy.[34,35]

In addition to different aged populations, the MF59-adjuvanted influenza vaccine showed better immunogenicity and sero-protection levels in human immunodeficiency virus (HIV)-infected patients[36,37] and chronic kidney disease patients undergoing hemodialysis[38], suggesting significant MF59 adjuvant effects in immunocompromised patients.

Until now, MF59 is licensed in influenza vaccines. There have been clinical trials to apply MF59 adjuvant to other vaccines covering bacteria and viruses. Human cytomegalovirus (HCMV) glycoprotein B subunit vaccine plus MF59 adjuvant conferred approximately 50% efficacy in preventing HCMV acquisition in a phase 2 trial[39] and its efficacy was mediated by non-neutralizing antibody functions.[40] MF59-adjuvanted *Staphylococcus aureus* vaccines induced protective humoral and cellular immune responses, CD4 T effector cell activity in mice.[41] MF59-adjuvanted recombinant vaccine containing Aventis Pasteur's canarypox vector–HIV gp120 increased immunogenicity to vaccine antigen in rhesus macaques although it could not delay the onset of simian immunodeficiency virus infection.[42]

MF59-adjuvanted vaccines prefer to induce Th2 immune-biased responses. The addition of TLR9 agonist CpG or TLR4 agonist E6020 to MF59-adjuvatned vaccines induced a more potent Th1 cellular immune response, which is represented by higher IgG2a titers and the induction of enhanced interferon-gamma response as well as similar or higher antibody titers.[43–45] HIV vaccines with MF59 plus Carbopol-971P (synthetic polyanionic carbomers) were shown to enhance binding and neutralizing antibody titers with higher avidity.[46,47]

Conclusion

MF59 is a safe and effective adjuvant licensed to be included in influenza vaccines. It is significant that MF59 can overcome a defect in CD4 T cell help in exhibiting adjuvant effects on enhancing adaptive immunity to vaccination, suggesting a new paradigm of CD4-independent adjuvant action mechanisms of MF59. This might explain adjuvant efficacy of MF59 in influenza vaccines in broader populations from young children to the elderly and in immunocompromised patients. It is expected that further studies will broaden the use of MF59 in various vaccine antigens and platforms as well as lead to better understanding of the mechanisms of MF59 adjuvant effects.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

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